TRBO-GT screen

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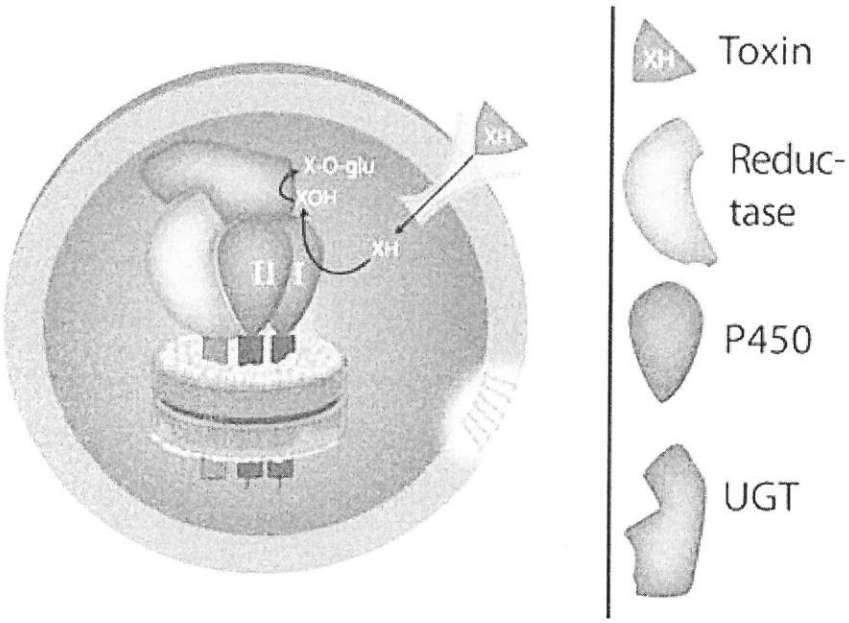

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***TRBO-GT screen**: Tobacco mosaic virus RNA Based Overexpressed-GlycosylTransferase assay and its application into 96 well format*

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Plant cell walls are composed mainly of polysaccharides, which not only have important roles for plant growth and development but also constitute the most abundant biomass component on the earth. The key enzymes for polysaccharide biosynthesis are glycosyltransferases (GTs). Approximately 450 GT genes in *Arabidopsis thaliana* have been identified based on their sequence, but the biochemical activity is unknown for most of the cell wall related GTs. To determine the function of a putative GT, it is essential to demonstrate activity of heterologously expressed protein. To identify novel GT activities, we have used transient overexpression of proteins in *Nicotiana benthamiana*, as a plant based expression system is more likely to produce active plant proteins than other common expression system based on yeast and animal cells.

To determine GT activities in a more efficient high throughput manner, we introduced three new strategies: 1) TRBO; ***T**obacco Mosaic Virus* **R**NA **B**ased **O**verexpression vector, 2) epitope tag purification of GTs synthesized in *Nicotiana benthamiana*, 3) GT assay in 96-well format to quantify transfer from NDP-sugar donors to exogenous acceptors. As for the target genes, about 300 GTs in Gateway vectors have been prepared. To select genes of highest priority, we used available expression data to identify GT genes that are highly expressed in woody tissue, which is important biomass. This gene selection allowed us to select specific GT genes for biomass accumulation from the large set of GT candidates in Arabidopsis and rice. In this study, we will present the methodology for high throughput TRBO screening of key cell wall GTs important for biomass accumulation.